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## Genetic Diversity in River Buffalo (*Bubalus bubalis*) Breeds of Central India using Heterologous Bovine Microsatellite Markers

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### Abstract

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A panel of 25 bovine specific heterologous microsatellite markers was employed for evaluating both within and between breed genetic variability estimates in Pandharpuri and Nagpuri buffaloes, two prominent breeds of Central India. A total of 148 alleles were detected across the two breeds with mean number of 5.92 alleles. The mean allelic diversity and heterozygosity reflected the existence of relatively higher genetic variability in Nagpuri buffalo. The F-statistics estimates were significantly different from zero ( $P < 0.05$ ) with  $F(F_{IT}) = 0.349 \pm 0.051$ ,  $f(F_{IS}) = 0.254 \pm 0.039$ ,  $\theta(F_{ST}) = 0.126 \pm 0.042$ . Most of the genetic variations correspond to differences among individuals (87.4%). Various genetic differentiation estimates indicated moderate differentiation between Nagpuri and Pandharpuri buffaloes. Individual assignment and inter-individual allele sharing estimates also substantiated the presence of discrete genetic structure in both the buffalo breeds from Central India.

Key words: Indian river buffalo, genetic diversity, microsatellite markers.

### Introduction

India is home to some of the best riverine buffalo (*Bubalus bubalis*), represented by ten well-defined breeds viz., Murrah, Niliravi, Bhadawari, Surti, Jaffarabadi, Pandharpuri, Marathwada, Mehsana, Nagpuri and Toda (Nivsarkar *et al.*, 2000). Besides these descript breeds, many lesser known buffalo populations are wide spread in the country each having its own special features and are

well adapted to their respective agro-climatic and eco-geographical conditions. Awareness of the value of genetic resources has encouraged studies on evaluating the genetic diversity present in different livestock breeds as an essential prerequisite to facilitate the conservation decisions. Therefore, genetic characterization is the first step to answer questions on taxonomy, evolution, domestication processes, management of genetic resources and conservation plans (Hall and Bradley, 1995). Several studies have successfully employed microsatellite markers to characterize the

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general genetic variation and population structure among breeds of various livestock species including buffaloes (Tantia *et al.*, 2006; Kumar *et al.*, 2006).

On similar lines, the present study was aimed to evaluate the extent of within breed genetic diversity and genetic differentiation between two buffalo breeds (Nagpuri and Pandharpuri) of Central India by utilizing 25 heterologous bovine specific microsatellite markers. Further, F-statistics, inter-individual level clustering based on proportion of shared alleles and breed assignment analysis were also performed to evaluate the genetic structure of these two important buffalo breeds.

### Materials and Methods

Blood samples were randomly collected from 47 genetically unrelated animals, each of Nagpuri and Pandharpuri buffaloes from their respective breeding tracts. Genomic DNA was extracted using standard phenol chloroform procedure of Sambrook *et al.* (1989). Twenty five heterologous bovine specific microsatellite markers were selected on the basis of their polymorphism status in buffaloes reported by Navani *et al.* (2002). PCR amplification of microsatellite loci was carried out in 25  $\mu$ l reaction volume with thermocycling conditions as: initial denaturation at 94C for 2 min, followed by 30 cycles of 1 min at 94C, 1 min at annealing temperature of each marker, 1 min at 72C. The final extension step was at 72C for 10 min. The PCR products were electrophoresed on standard 6% Urea-PAGE denaturing sequencing gel and resolved bands of DNA (alleles) were visualized by silver staining procedure of Bassam *et al.* (1991). Allelic size range was estimated using 10 bp ladder (Invitrogen, Life Technologies, USA) run in parallel with the samples. Genotype of individual animals of the two breeds at 25 microsatellite loci was recorded by direct counting.

POPGENE software package (Yeh *et al.*, 1999) was used to calculate allele frequencies, observed number of alleles ( $N_o$ ), effective number of alleles ( $N_e$ ), observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ). The test for departure from Hardy Weinberg proportions was performed using exact probability tests provided in GENEPOP version 3.1d. (Raymond and Rousset, 1999). Monte Carlo method (Guo and Thompson, 1992) was applied to compute unbiased estimates of the exact probabilities (P-values). F-statistics parameters of  $F$  ( $F_{IT}$ , total inbreeding estimate),  $\theta$  ( $F_{ST}$ , measurement of population differentiation) and  $f$  ( $F_{IS}$ , within-population inbreeding estimate) were computed using FSTAT version 2.9.3.2 computer programme (Goudet, 2002). Various genetic distances among populations namely Nei's  $D_s$  (1972); Nei's  $D_A$  (1983) and Goldstein's  $(\delta\mu)^2$  distance (1995) was estimated from the allele frequencies data. Using the allele-sharing ( $D_{AS}$ ) inter-individual genetic distance matrices (Bowcock *et al.*, 1994), with individual animal acting as operational taxonomic unit (OTU), cluster analysis of two buffalo breeds was inferred in the form of N-J algorithm based radiation tree using Drawtree programme of PHYLIP package (Felstenstein, 1993). The multi-locus genotypes of individuals were also evaluated in an assignment assay as a measure of genetic distinctness between the two breeds using Gene Class V.1.0.02 software (Piry *et al.* 2004).

### Results and Discussion

The number of observed alleles ( $N_o$ ) ranged from 2.00 (ILSTS031, ILSTS073) to 8 (HEL13) with an average of 4.6 alleles in Pandharpuri and 2.00 (ILSTS073) to 8.00 (ILSTS058, HEL13) with an average of 5.3 alleles in Nagpuri buffaloes (Table 1). A total of 148 alleles were detected across the two buffalo breeds with mean number of 5.92 alleles (MNA). Effective number of alleles ( $N_e$ ) in

Table 1  
 Microsatellite alleles ( $N_O$ -Observed,  $N_E$ -Effective), heterozygosity ( $H_O$ -observed,  $H_E$ - expected)  
 and  $F_{IS}$  in two buffalo breeds at each locus

Locus	Pandharpuri					Nagpuri				
	$N_O$	$N_E$	$H_O$	$H_E$	$F_{IS}$	$N_O$	$N_E$	$H_O$	$H_E$	$F_{IS}$
CSRM60	6.0	2.7	0.533	0.638	0.165*	7.0	2.4	0.268	0.588	0.547*
ILSTS026	4.0	1.8	0.409	0.446	0.083	5.0	2.0	0.512	0.501	-0.022
HEL13	8.0	4.3	0.667	0.778	0.145*	8.0	4.8	0.698	0.802	0.131*
ILSTS030	5.0	2.4	0.487	0.592	0.180*	4.0	2.2	0.488	0.556	0.122*
ILSTS033	5.0	3.9	0.691	0.755	0.087	5.0	2.6	0.488	0.616	0.209*
ILSTS017	4.0	3.6	0.651	0.731	0.111*	5.0	3.1	0.591	0.687	0.141*
ILSTS019	3.0	1.2	0.200	0.186	-0.079	3.0	1.1	0.065	0.105	0.384*
ILSTS045	3.0	2.3	0.140	0.569	0.757*	4.0	1.4	0.286	0.291	0.019
ILSTS034	3.0	1.4	0.046	0.314	0.856*	4.0	1.4	0.234	0.288	0.189*
ILSTS058	7.0	4.8	0.317	0.800	0.607*	8.0	6.1	0.341	0.846	0.600*
ILSTS056	4.0	1.3	0.256	0.255	-0.002	6.0	1.4	0.319	0.290	-0.103
ILSTS089	5.0	4.5	0.400	0.790	0.497*	6.0	4.6	0.750	0.793	0.055*
CSSM66	5.0	3.0	0.714	0.670	-0.068	4.0	2.6	0.610	0.622	0.019
ILSTS036	4.0	2.4	0.171	0.592	0.714*	6.0	3.4	0.404	0.714	0.436*
ILSTS095	6.0	2.1	0.379	0.540	0.302*	5.0	2.6	0.357	0.618	0.425*
ILSTS029	3.0	1.6	0.129	0.393	0.677*	5.0	1.6	0.378	0.368	-0.027
ILSTS028	5.0	3.2	0.435	0.696	0.378*	6.0	4.5	0.711	0.788	0.099
ILSTS025	5.0	3.1	0.500	0.685	0.272*	7.0	5.0	0.535	0.811	0.343*
ILSTS052	7.0	3.6	0.450	0.732	0.388*	7.0	4.7	0.581	0.795	0.271*
ILSTS031	2.0	1.4	0.311	0.266	-0.173	4.0	2.0	0.691	0.517	-0.340
ILSTS073	2.0	1.9	0.455	0.468	0.029	2.0	1.9	0.349	0.479	0.273*
ILSTS060	4.0	3.0	0.841	0.680	-0.240	4.0	2.3	0.389	0.576	0.328*
BM1818	5.0	4.0	0.568	0.760	0.255*	5.0	2.6	0.382	0.619	0.386*
ILSTS061	6.0	2.8	0.404	0.652	0.382*	7.0	3.7	0.444	0.739	0.402*
ILSTS068	5.0	3.3	0.432	0.703	0.388*	5.0	4.2	0.513	0.772	0.338*
<b>Mean</b>	<b>4.6</b>	<b>2.8</b>	<b>0.423</b>	<b>0.588</b>	<b>0.282*</b>	<b>5.3</b>	<b>2.9</b>	<b>0.455</b>	<b>0.591</b>	<b>0.232*</b>

\*Values significant at  $P < 0.05$ .

two buffalo breeds was distinctly less than the observed values across all loci. The overall allelic diversity observed in the two breeds was similar to other Indian buffalo breeds observed by Tantia *et al.* (2006). The average observed heterozygosity ( $H_O$ ) in the two breeds was less than the average expected heterozygosity. There was significant deviations from HWE ( $P < 0.05$ ) at

several loci in both the populations. The basis of this departure could be linked to fairly high positive  $F_{IS}$  (within-population-inbreeding estimate) values obtained in both the buffalo breeds. Population wise, significant deficit of heterozygotes ( $F_{IS}$ ) was observed in both the breeds (Table 1). The mean estimates for  $F$ -statistics in the two Indian buffalo population across 25 loci were

significantly ( $P < 0.05$ ) different from zero:  $F (F_{IT}) = 0.349 \pm 0.051$ ,  $f (F_{IS}) = 0.254 \pm 0.039$ ,  $\theta (F_{ST}) = 0.126 \pm 0.042$ . This  $F$ -statistics data indicated the existence of genetic homogeneity and population structure in the two analyzed buffalo breeds. The deficit of heterozygotes ( $F_{IS} > 0$ ) observed in the two buffalo breeds could be attributed to the factors like assortative mating (sample relatedness), linkage with loci under selection (genetic hitchhiking), population heterogeneity or the null alleles.

The overall value of classical multi-locus genetic differentiation estimator:  $F_{ST}$  (0.126) reflected moderate degree of differentiation between the two buffalo breeds. The present analysis fractionated most of the genetic variation to the differences among individuals (87.4%) while only 12.6% corresponds to the differences among breeds. The overall  $N_m$  values averaged 1.73, indicating low rate of gene flow between the Pandharpuri and Nagpuri buffaloes. The  $P$  values of exact test differed significantly ( $P < 0.001$ ) from zero at most of the loci (data not shown). The interbreed relationship evaluated through genetic distance measures *viz.*  $D_S$  (0.253),  $D_A$  (0.170),  $\delta\mu^2/(1.89)$  further supported the substantial genetic divergence between the two buffalo breeds. Further assignment test generated near perfect assignments scores of the individuals to their respective population, reflecting the limited gene flow between the two breeds. The radiation tree based on proportion of allelesharing distance among the Nagpuri and Pandharpuri individuals produced tight clusters with two monophyletic groups coinciding with the respective source population (Fig. 1). The various inter-breed analysis yielded the presence of discrete genetic structure in both the buffalo breeds which corroborated the findings of Kumar *et al.* (2006) where separate clustering of Nagpuri and Pandharpuri buffaloes has been reported.

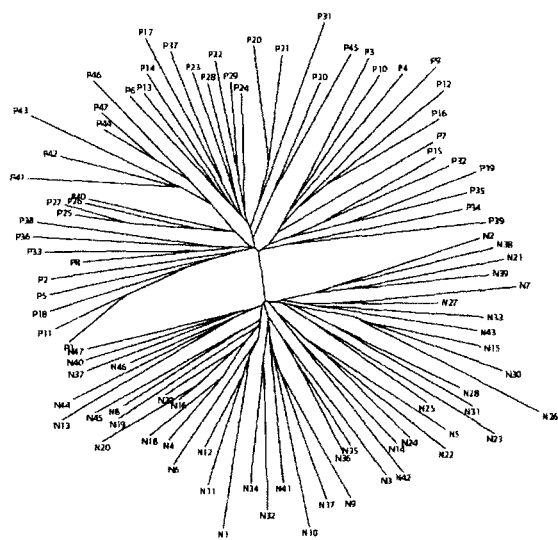


Fig. 1. Radiation tree from inter individual distance using allele sharing method showing distinct cluster of Pandharpuri(P) and Nagpuri(N) buffaloes.

The study thus indicated valuable insight into the genetic structure of the two important buffalo breeds of Maharashtra state of Central India. Our results further validate the usefulness of the present set of 25 bovine specific microsatellite markers to evaluate the genetic structure of Indian buffalo breeds

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बी.पी. मिश्र, आर.एस. कटारिया, एस.एस. बुलन्दी, वी. कुमार, एम. मुकेश । विषमजात गोवंशी माइक्रोसेटेलाइट मार्करों के उपयोग से मध्य भारत की जलीय भैंसों ( बुबेलस बुबेलिस ) में आनुवंशिक विविधता ।

मध्य भारत की दो महत्वपूर्ण नस्लों, पंढरपुरी और नागपुरी भैंसों में अंतःनस्ली और अंतरनस्ली विविधता आंकलन के लिए 25 गोवंशी विशिष्ट विषमजात माइक्रोसेटेलाइट मार्करों के एक समूह का उपयोग किया गया । औसत 5.92 एलिलों के साथ दोनों नस्लों के 148 एलिलों को ज्ञात किया गया । औसत एलिली विविधता और विषमजातता से नागपुरी नस्ल में अधिक सापेक्ष आनुवंशिक विविधता पाई गयी । एफ सांख्यिकी आंकलन शून्य के साथ एफ (  $F_{आईटी} = 0.349 \pm 0.051$ , एफ (  $F_{18} = 0.254 \pm 0.039$ , (  $F_{एसटी} = 0.126 \pm 0.042$  ) का सार्थक अंतर था । अधिकांश आनुवंशिक विविधताएं ( 87.4% ) वैयक्तिक भेदों से थीं । विभिन्न आनुवंशिक विभेदन आंकलन ने पंढरपुरी और नागपुरी के मध्य थोड़ा विभेदन दर्शाया । वैयक्तिक निर्दिष्टीकरण और अंतरवैयक्तिक एलिल भागीदारी आंकलनो से भी केन्द्रीय भारत की भैंस की दोनो नस्लों में विविक्त आनुवंशिक संरचना को बल मिला ।